APPLICATION NOTE

Genetic Toxicology Testing Services

FRONTAGE
YOUR DRUG DEVELOPMENT PARTNER

Genotoxicity assessment is an essential component of the safety assessment of all types of substances, ranging from pharmaceuticals, industrial chemicals, pesticides, biocides, food additives, cosmetics ingredients, to veterinary drugs, relevant in the context of international legislations aiming at the protection of human and animal health.

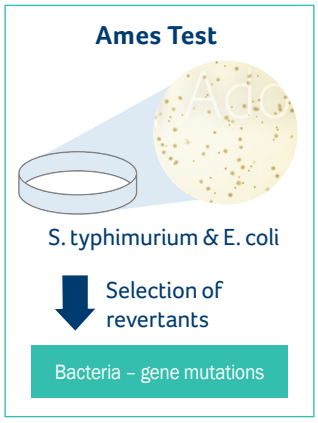
In general, the assessment of genotoxic hazard to humans follows a step-wise approach, beginning with a basic battery of In-Vitro tests followed in some cases by In-Vivo testing.

Frontage offers the portfolio of assays and designs to meet all regulatory requirements for IND in Genetic Toxicology testing.

- Bacterial reverse mutation test (OECD TG 471)
  - Accurate, robust, reproducible, and customizable
- In-Vitro mammalian cell micronucleus test (OECD TG 487)
  - Using TK 6 cell lines
- In-Vivo mammalian erythrocyte micronucleus test (OECD TG 474)
  - In Rats and Mice (Blood and Bone Marrow)

Bacterial Reverse Mutation Test (OECD TG 471): Ames Test (GLP & Non-GLP)

The bacterial reverse mutation test is the most widely used assay to detect gene mutations. The test uses amino acid requiring strains of Salmonella typhimurium (TA98, TA100, TA1535 & TA1537) and Escherichia coli (WP2) to detect mutations, which involve substitution, addition, or deletion of one or a few DNA base pairs. It has the ability to differentiate between frame-shift and base-pair substitutions with the use of different bacterial strains.

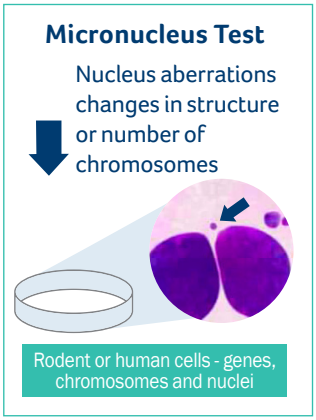


The principle of this test is to detect revertant bacterial colonies with restored functional capability to synthesize an essential amino acid, histidine for S. typhimurium strains and tryptophan for *E. coli* strain. Bacteria are exposed to the test substance both in the presence and absence of an appropriate metabolic activation system (post-mitochondrial fraction: S9).

The revertant bacteria are detected by their ability to grow in the absence of the amino acid required by the parent test strain. The bacterial reverse mutation test is rapid, inexpensive and relatively easy to perform. The number of strains used for Non-GLP studies are two (TA98 & TA100) or vary based on sponsor request.

In-Vitro Mammalian Cell Micronucleus Test (OECD TG 487): Using TK6 Cell Lines

The in-Vitro mammalian cell micronucleus test (MNvit) is used to identify substances that cause structural and numerical chromosomal damage in cells that have undergone cell division during or after the exposure to the test substance.



The assay detects micronuclei in the cytoplasm of interphase cells and typically employs human or rodent cell lines or primary cell cultures.

The In-Vitro mammalian cell micronucleus test can be conducted in the presence or in the absence of cytochalasin B (cytoB), which is used to block cell division and generate binucleate cells. Cultured primary human or other mammalian peripheral blood lymphocytes and a number of rodent cell lines such as CHO, V79, and L5178Y cells or human cell lines such as TK6 can be used in presence and absence of appropriate metabolic activation system (post-mitochondrial fraction: S9).

Appropriate techniques should be used to control any bias or drift when using an automated scoring system like flow cytometry. The MNvit is rapid and easy to conduct and it is the only In-Vitro test that can efficiently detect both clastogens and aneugens.

In-Vivo Mammalian Erythrocyte Micronucleus Test (OECD TG 474): In Rats and Mice (Blood and Bone Marrow)

In-Vivo mammalian erythrocyte micronucleus test (MNviv) is to identify substances that cause structural and numerical chromosomal damage in somatic cells In-Vivo. The damage results in the formation of micronuclei, containing chromosome fragments or whole chromosomes in young (polychromatic) erythrocytes sampled in bone marrow and/or reticulocytes of peripheral blood cells of animals, usually rodents. Mice, rats, or another appropriate mammalian species may be used.

When peripheral blood is used, it must be established that splenic removal of micronucleated cells from the circulation does not compromise the detection of induced micronuclei in the species selected. This has been clearly demonstrated for mouse and rat peripheral blood. Most studies could be performed in either sex as micronucleus response is similar between male and female animals. The frequency of micronucleated immature erythrocytes is the principal endpoint. Appropriate techniques should be used to control any bias or drift when using an automated scoring system like flow cytometry. The MNviv is still the most widely used In-Vivo genotoxicity test that detects both clastogens and aneugens.

Frontage Laboratories, Inc. is a contract research organization (CRO) that provides integrated, science-driven, product development services throughout the drug discovery and development process to enable pharmaceutical and biotechnology companies to achieve their development goals. Comprehensive services include drug metabolism and pharmacokinetics, analytical testing and formulation development, preclinical and clinical trial material manufacturing, bioanalysis, preclinical safety and toxicology assessment and early phase clinical studies. Frontage has enabled many biotechnology companies and leading pharmaceutical companies of varying sizes to advance a myriad of molecules through development and file regulatory submissions in the United States, China, and other countries.

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